Fibre Diffraction Experiments with Micron-Sized Beams

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Scanning diffractometry with beam sizes down to about 2 microns can be used to map hierarchical structures such as polymer fibres or biopolymers. Instrumentation developed at the ESRF microfocus beamline is based on an undulator source, a double focusing mirror and post collimation by collimators or glass capillaries. The method has been applied to a range of topics from spherulitic structures to human hair. Recent instrumental developments suggest that SAXS and WAXS experiments can be performed using a very simple single detector setup. Due to the focusing of the beam, the SAXS resolution is, however, limited to about fifty nanometers. In principle the high flux density allows the investigation of single crystals or very highly textured samples which were accessible until now only to fibre diffraction. Typical cases are chitin and amylose. Techniques developed to study such samples will be discussed.

Tobacco Mosaic Virus as a Model for Phasing and Refinement in Fibre Diffraction

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Tobacco mosaic virus (TMV) has the largest asymmetric unit of all structures solved so far by fibre diffraction at atomic resolution. TMV is exceptionally stable, and easily oriented. For these reasons, it has been a model for the development of fibre diffraction methods for more than sixty years. The first diffraction patterns from TMV, and the first recognizably non-crystalline fibre diffraction patterns, were obtained by Bernal and Fankuchen in 1936.

TMV was the first fibre diffraction system in which the isomorphous replacement method was used, only one year after its first use in protein crystallography. Franklin and Caspar independently determined the radial density distribution of the virus; twenty years later we used a multi-dimensional analysis of data from six heavy atom derivatives to solve the cylindrically averaged phase problem.

In the past 15 years, TMV has been a model for the use of macromolecular crystallographic refinement methods in fibre diffraction. Restrained least-squares methods were adapted and used to refine the structure of TMV; molecular dynamics methods were later used to refine several other tobamovirus structures. Current work is focused on developing methods of refining structures against undeconvoluted data, in which the disorientation is too great to allow separation of layer line intensities.

High Resolution Definition of the Axial Molecular Packing of Fibrillar Type I Collagen. A Model Independent Phase Determination

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A high resolution definition of the projected axial structure of collagen presents detailed information about the conformation of the non-helical telopeptides, the relative ratio of the gap/overlap period, and the axial alignment of the collagen molecules within the microfibril. The information needed to define such a structure is contained within the meridional Bragg reflections. However, the majority of previous studies of collagen structure, whether utilising this information or not, have been negatively biased by their model dependent nature. It has been demonstrated in this study, that it is possible to phase the meridional diffraction pattern of type I collagen in an unambiguous way through isomorphous addition. Diffraction data have been recorded using synchrotron radiation sources, and isomorphous derivative proteins have been made

with the minimum of labelling sites. Over 100 meridional intensities have been used to produce a one dimensional electron density map of a single D-repeat of the collagen fibril. From this, the conformation of the N and C telopeptides have been determined within the tight constraints allowed by the derivative labelling shown through difference Fouriers and the 1-D real space model of the native protein. It has been shown that the five collagen chains are equally aligned each containing 234.2 amino acids within the axial unit cell, and that the C-terminal telopeptide is in a folded conformation whilst the N-terminal telopeptide is contracted.

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Fibrillin: A Basis for Tissue Elasticity and Recoil

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Fibrillin is an extracellular protein found to be present in many locations where elasticity is required. It is found in almost pure form in the zonular filaments of the mammalian eye. Molecular assemblies of fibrillin molecules form beaded structures that exhibit apparent variability which may be responsible for the elastic response. This was tested by X-ray diffraction since the changes in the fundamental periodicity of the fibrillin microfibrils would be shown by changes in the meridional diffraction pattern. X-ray diffraction revealed a low angle pattern consisting of eight low angle meridional reflections. The intensity distribution was found to change with the removal of calcium by chelation. The changes in the fundamental period were also not straightfoward. Tissues such as zonular filaments appear to contain two types of fibrillin microfibril packing. Firstly, there are regions of well defined molecular stagger that act as molecular junctions maintaining the tissue integrity, with secondly regions of variable bead length that only make a contribution to diffraction when calcium is removed.

Squeezing Information From Non-Crystalline Diffraction

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The advent of X-ray synchrotron radiation sources has dramatically increased the range and detail which can be obtained in X-ray scattering studies of partially ordered materials. This presentation will illustrate these development with examples from following areas:

(i) characterisation of pathways in strain-induced crystallisation of polymer materials;

(ii) structural variation in polymer materials at high spatial resolution;

(iii) correlation of stress-strain data with small angle scattering in micro-deformation of polymer materials;

(iv) effect of various protocols for biaxial drawing of polymer materials on molecular organisation.

Hard X-Ray Microscopy: Micro-Diffraction, Imaging and Spectroscopy with Coherent Synchrotron Radiation

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The last several years have seen a tremendous breakthrough in the development of microoptics for high energy X-rays. The very low divergence and source sizes of the ESRF beams allow efficient focusing down to a submicron spot sizes using Bragg-Fresnel Optics, Fresnel Zone Plates and Compound Refractive Lenses. Recently

commissioned at the ESRF on ID 22, the Micro-fluorescence, imaging and diffraction (μ-FID) beamline is devoted to the study of samples at the micron and submicron scale in the energy range from 5 to 60 keV. A new flexible setup has been designed to allow the use of the beam after a system of flat mirror and fixed-exit vertically deflecting double-crystal monochromator. Optical components were carefully optimized to conserve the coherence properties of the beam, allowing the observation of holographic patterns of low-density materials. All probes can be used either in the single spot or mapping mode with a sub-μm scanning precision. The methods of investigation comprise microdiffraction (SAXS, WAXS), spectroscopy (XRF, XAS, fluorescence tomography) and imaging (phase contrast imaging/tomography, holography, topography, interferometry). The availability of the hard X-ray microscopy techniques is opening up research opportunities for a broad range of disciplines from semiconductor device engineering to biomedical applications. Results from enviromental, medical and biological sciences will be presented.

Analysis of Time-Sequence Data with FIT2D

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High brilliance 3rd generation synchrotron radiation sources combined with the collection efficiency of on-line area detectors mean that time-resolved scattering and diffraction experiments generate large quantities of data. Automated analysis of these data is highly desirable. User interaction, however, remains important to allow versatile masking of contaminating features, etc.

In FIT2D the "FILES SERIES" option "INTEGRATE" has been developed to allow the combination of interactive data analysis and subsequent automated analysis. Raw data may optionally be corrected for detector distortions, and integrated to 1-D 2-theta scans with user defined masking. After successful integration of the first image the integration is repeated automatically for subsequent images.

Small-Angle Diffraction from Mesophases in Colloidal Dispersions of Plates

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Small-angle scattering of X-rays and neutrons has been used to investigate the structure in dispersions of plate-like colloidal particles. A model system of monodisperse hexagonal plates of $Ni(OH)_2$ has been prepared. It can be stabilised with polyacrylate to provide almost hard particle interactions. The diffraction pattern has allowed us to show that at high concentrations there is a columnar phase and, at lower concentrations, there is a region of coexistence with a less ordered phase. The possibility of a cubic phase as an intermediate state before an isotropic liquid phase is found at the lowest concentrations is being investigated. The benefits of SAXS and SANS for different aspects of the study will be mentioned. Work on shear alignment of the structures has led to an observation of a phase change to a layer structure.

Transient Liquid Crystallinity in the Drawing of Polyesters

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The discovery of a transient smectic phase in random co-polymers of PET and PEN in samples quenched while they were being drawn opened up new levels of understanding and new lines of enquiry:

(a) The slower crystallisation of the random copolymers made the experiment more easy than with the parent homopolymers.

(b) The same effect was observed in the parent homopolymers, although the temperature window from which the quench had to be

performed became more critical. It provided compelling confirmation of previous, somewhat scattered, reports.

(c) Collaboration with Watson Fuller and his group enabled on line confirmation of the appearance of the phase.

(d) The conversion of the smectic phase to the crystalline phase in PET rich members of the series, provides an explanation of the cause of the so-called c-tilt effect well known in PET.

(e) The different response of PEN rich samples to the smectic/crystal transition, provides an explanation of the continuous layer line background, well recognised as a characteristic of the crystalline pattern, while at the same time accounting for the absence of c-shear in this class of sample.

The paper will discuss the findings in the context of on going work.

Reaction Induced Phase Separation: Smart Polymer Processing

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Poly(2,6-dimethyl-1,4-phenylene ether) (PPE) is difficult to process without the use of solvents. PPE was dissolved in epoxy resin and reaction induced phase separation in the blend was studied using a time-resolved, small-angle light-scattering camera equipped with an optical DSC. Laser light scattering measurements characterize the subsequent spinodal decomposition process. A four-stage model is discussed: (a) onset of phase separation, (b) early stage of spinodal decomposition, (c) late stage of spinodal decomposition, and (d) apparent phase dissolution. Cahn-Hilliard linear theory, yielding initial correlation lengths and effective interdiffusion coefficients, accounts for the early stage of the spinodal decomposition. In the late stage, the scattering peak maximum, q_m, starts to decrease with time according to a power-law. However, the maximum in the scattered intensity, I_m, does not satisfy a power-law due to the large change in the epoxy refractive index during crosslinking. The phase-inverted morphology and mechanical properties of the cured blend are investigated by SEM, TEM and DMTA.

Neutron Scattering Studies of the Effect of Ca^{2+} on the *In Situ* Structures of Troponin I and Troponin C

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Neutron small-angle scattering using contrast variation is a powerful method to determine the quaternary structure of biological macromolecular complexes. If the complexes have natural internal contrast due to having components of different chemical composition (*e.g.* proteins and nucleic acids) then contrast matching can be attained through the use of appropriate H_2O/D_2O mixtures. In cases where this is not so then deuteration of parts of the complex by disassembly and reassembly of specifically *in vivo* deuterated components can be used. We have used this latter technique to investigate the *in situ* structures of troponin C (TnC) and troponin I (TnI) in whole troponin and the influence of regulatory amounts of Ca^{2+} on these structures. In separate difference experiments, 97% deuterated TnC and TnI within whole troponin were studied with and without Ca^{2+} in 41.6 mole % buffers in which protonated sub-units are invisible. The radius of gyration (R_g) of TnI was found to decrease by ~10% on addition of regulatory calcium indicating a significant compaction of the structure. The cross-sectional radius of gyration (R_c) increased by ~9% under the same conditions. Modelling

studies showed that the high-Q scattering could be fit by a TnI molecule consisting of two domains; one a highly oblate ellipsoid of revolution containing about 65% of the mass, the other a highly prolate ellipsoid. Similar experiments with deuterated TnC demonstrated that it was elongated *in situ* and that its radius of gyration was not sensitive to the Ca^{2+} occupancy of its regulatory sites. The cross-sectional radius of gyration did increase with Ca^{2+} addition in a manner expected from crystallographic and NMR studies of the isolated TnC.

X-Ray Diffraction Probing of Molecular Events During Muscle Contraction

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Although there are several distinct biological molecular motors, striated muscle has great advantages in structural studies since the highly oriented, force-producing filaments actin and myosin are distributed regularly and at high concentration through macroscopic volumes of tissue [1]. It is therefore possible with muscle, as with no other molecular motor, to study the structural behaviour of the interacting molecules in a fast, time-resolved mode, using synchrotron radiation, optimised low-angle beam-lines and state of the art X-ray detectors [2]. Currently, the Daresbury SRS, low-angle beam-line 16.1, and the RAPID multiwire area detector together provide a world-beating combination. Although the SRS is no longer a state of the art source, no third generation synchrotrons, such as ESRF, SPring8 or APS, have detectors that are close in their count-rate capabilities to RAPID. When the UK's new DIAMOND synchrotron is a reality, beamlines equivalent to 16.1 together with RAPID detectors will permit time-resolved X-ray diffraction studies of dynamic biological (and other) systems to be carried out with unprecented accuracy and reliability. In the present study we have been attempting to monitor the sequence of events involved in muscle activation and regulation [2,3,4]. In a typical experiment a highly ordered 'fin' muscle from plaice is electrically stimulated at time zero with a 50 Hz AC field to give a sustained tetanic contraction. During the roughly 150 to 200 ms tension rise time the muscle's low-angle diffraction pattern is monitored in 1 ms time bins. This is compared with longer exposures both before activation and at the tension plateau of the tetanus. Tension relaxation following cessation of the stimulus field was monitored in 2 ms time bins. The general structure of this muscle [1,3] and the molecular changes involved in the control and production of force in the plaice contractile cycle will be described [2,4]. In particular the change of the equatorial (1,1) reflection is rather fast, compared with the changes of the equatorial (1,0) reflection and tension, which have about the same time-courses. The meridional 14.3 nm reflection changes at an intermediate rate. The relative speed of the change of the actin second layer-line, indicative of activation events, is being determined and will be described.

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Unit Cell Structure and Intermolecular Interactions in *Lethocerus* Fibrillar Insect Flight Muscle in Defined States

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In the interest of studying the molecular structure and function of muscle, we are trying to determine the

3D structure of actin and myosin filaments and the structural changes underlying contraction and its regulation. Our main approach involves using methods developed in a BBSRC-funded study of fish muscle (Hudson *et al.*, 1997). Here these methods are being used to solve the full unit cell structure in relaxed insect flight muscle and also rigor insect muscle. Actin molecules and myosin heads are particularly well organized in insect flight muscles and give rise to semi-crystalline low-angle X-ray diffraction patterns.

Our group at Imperial College have already analysed and solved the structure of myosin filament in relaxed insect flight muscle (Hudson *et al.*, in preparation). We are now refining this structure and propose to apply these methods to solve the full unit cell, including the actin filaments with troponin and tropomyosin, in both relaxed and rigor insect flight muscle using new X-ray diffraction data. This will provide direct information on the actin-myosin interface and also on the conformation and the flexibility of the muscle heads in different defined sates.

Initial studies (Hudson *et al.*, in preparation) on modelling the structure of the myosin filament in relaxed insect flight muscle to 7.0 nm resolution using the myosin head shape of Rayment *et al.* (1993) and the simulated annealing procedure of Hudson *et al.* (1997), gave a crystallographic R-factor of 5.97 % against 65 reflections with the resting crossbridges projecting at 90° to the filament long axis as originally reported by Reedy *et al.* (1965).

In this meeting, we will present a poster showing our latest results on this project.

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X-Ray Camera for Wide-Angle X-Ray Diffraction Studies of the Biaxial Deformation of Polymers

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An X-ray fibre diffraction camera capable of biaxial deformation of polymer films, has been developed in the Physics Department of Keele University. The camera allows time-resolved X-ray studies of the change in orientation and crystallinity at draw rates of up to $12s^{-1}$ and draw ratios of up to 6:1 to be recorded. The application of the camera in the study of biaxial deformation of poly(ethylene terephthalate) (PET) during experiments on synchrotron and laboratory sources is described.

An Age-Dependent Study on the Effects of Photorefractive Keratectomy on Rabbit Cornea, using Transmission Electron Microscopy and X-Ray Diffraction Techniques

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Corneal transparency is due to the uniformly small diameter collagen fibrils and to the high degree of ordering in their lateral arrangement. Photorefractive keratectomy (PRK) is a surgical technique in which a laser is used to re-shape the front surface of the cornea and thus produce a refractive change to compensate for the effects of (usually) myopia. Previously, corneal haze following PRK has been studied using young rabbits. To look for an age-

related response, we have measured haze in young (2.5kg) and old (5kg) rabbits. The right eyes of 8 rabbits were subjected to PRK using an argon-fluoride excimer laser (193nm). Objective measurements of haze were made at various time intervals during wound healing. The anterior stroma (0-2μm below the epithelium) was studied to predict the transmission of visible light through the wounded corneas. The haze measurements showed that old rabbits had an increased response that peaked at 23 days post-surgery, but over the longer term (12 months) the haze levels were similar. Electron microscopy revealed that, 8 months after surgery, there was a large zone, up to 2μm thick, of irregularly spaced and poorly oriented collagen fibrils directly below the epithelium. X-ray diffraction measurements were used to determine the fibril refractive index and to scale up electron micrographs to account for shrinkage during preparation for microscopy. The relative positions and diameters of the individual fibrils were obtained from the scaled micrographs and the Direct Summation of Scattered Fields model (Freund, D.E. *et al., Appl. Optics* (1986) **25**, 2739-2746) was used to predict the transmission of visible light through the anterior of the cornea. There was no significant predicted increase in light scattering in either young or old rabbits following PRK. Disorder in the newly deposited collagen does not, therefore, explain the observed post-operative haze.

The Micro-SAXS Facility at the ESRF-Beamline ID22

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ESRF beamline ID22 is designed for microscopic studies of material's properties with hard X-rays. Basic elements are focusing and imaging optics through which sub-micrometer resolution is obtained. Besides direct imaging and various micro-spectroscopic methods, micro-diffraction fits into the beamline activities. In this context a micro-SAXS camera has been set up recently and has been tested in a series of experiments.

The micro-SAXS camera consists of a Fresnel Zone plate, a collimating system and CCD-based area detectors. X-rays of energy around 10 keV are focused to 2 μm (vertical) by 10 μm (horizontal) at the sample position with a flux of 5×10^{10} ph/s. The lens-sample distance is 1300 mm. The divergence after the lens is 5×10^{-4} yielding a resolution in momentum transfer of 0.01 nm^{-1}. Currently the collimator limits the minimum momentum transfer to 0.05 nm^{-1}. Two 2D detectors are available with a DQE of 0.5 (0.7) and an active area of 35 mm (100 mm) diagonal. The pixel size is 23 μm (58 μm), the dynamic range is 16 bit. The detectors have a dark-noise of 100 counts/pixel. The radiation background is in the range of the detector's noise. The sample-detector distance can be varied between 30 mm and 2m. Sample alignment can be accomplished with an optical microscope online. Because of suitable lens-sample distances, samples can easily be changed and handled.

With this micro-SAXS camera, local diffraction patterns of collagen fibres (rat-tail tendon) have been collected. Significant intensity of 1st to 20th diffraction orders was collected in a 30 seconds exposure. Scattering from diluted calcification volumes along the mineralisation direction of growing bone was recorded with micrometer spatial resolution.

In future the detectable minimum momentum transfer will be improved.

Templated Crystallisation: Soft Phases Controlling Hard Materials

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Throughout nature, the structure of hard materials *e.g.* coral, is controlled by a soft template. We have examined crystallisation in shear oriented block

copolymers, where a soft phase of microphase separated melt controls the preferred direction of crystallisation. In lamella phases, the comparison is simple and direct over a range of molecular weight and domain sizes. For hexagonally arranged cylindrical systems and for cubic gyroid structures, the choice of preferred directions is limited. We have used real time SAXS to monitor the structure formation upon crystallisation. Results will be presented for recent measurements on stations 16.1 and 2.1 and at DUBBLE at the ESRF.

Crystallization and Morphology Development in Oriented Poly(Ethylene Terephthalate) and Related Copolymer Films

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The molecular morphology of several oriented poly(ethylene terephthalate) (PET) and copolymer films have been investigated using Small- and Wide-angle X-ray Scattering (SAXS/WAXS) techniques at the Daresbury Synchrotron Radiation Source (SRS). The films were uniaxially deformed in a purpose built instrument to mimic typical industrial processing conditions. During sample deformation, synchronized SAXS/WAXS data were collected using a 2-D gas-filled multiwire area detector and a portable CCD based area detector respectively.

The samples were deformed at a rate of 24000% min^{-1} at 85°C, then step annealed up to a temperature of 220°C. The 2-D X-ray data obtained were used to explore the morphological changes in the samples as annealing progressed. The 2-D SAXS data were used to determine detailed information pertaining to the samples' changing crystallinity and lamellar structure. This information was obtained by using a purpose written 1-D correlation function analysis package known as *corfunc*. The X-ray data were also coupled with physical density measurements of the films to ascertain their crystallinity as a comparative method.

From the X-ray and physical analysis of the PET films deformed under industrial processing conditions, it has been possible to evaluate the changes in crystallinity and lamellar structure during annealing. The results indicate that the homopolymer PET sample displays differences in the development of crystallinity and lamellar structure compared to that of the copolymer samples. Generally, the homopolymer sample has increased crystallinity compared to certain copolymer samples, leading to initial conclusions that the addition of only a small fraction of comonomer has the ability to significantly change the morphology of a PET film during processing.

Effect of pH and Calcium, Phosphate and Phosphopeptide Concentrations on the Size and Substructure of Calcium Phosphate Nanoclusters as Determined by X-Ray and Neutron Scattering and Circular Dichroism Spectroscopy

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The ability of casein in the form of colloidal-sized casein micelles to modulate the phase separation of calcium phosphate during milk secretion was adapted to produce nm-sized particles of calcium phosphate stabilised by a 25 amino acid N-terminal tryptic phosphopeptide of bovine ß-casein (nanoclusters). The nanoclusters were prepared from an undersaturated solution of salts and the peptide by raising the pH homogeneously from about 5.5 to 6.7

with urea plus urease. Chemical analysis, multinuclear nmr and infrared spectroscopy showed that they comprise an amorphous dicalcium phosphate bound to the phosphopeptide.

Calcium phosphate nanoclusters were prepared under standardised conditions using 10 mg ml^{-1} of the phosphopeptide as stabilising agent. The molecular mass determined by sedimentation equilibrium was 197,600±13,700 and the apparent radius of gyration determined by X-ray scattering was 2.80±0.05 nm. A small angle neutron scattering contrast variation study in $1H_2O$-$2H_2O$ mixtures was performed and gave radii of gyration at the calculated match points for the calcium phosphate (88.2% $2H_2O$) and phosphopeptide (41.3% $2H_2O$) of 3.39±0.08 nm and 1.85±0.05 nm respectively. Measurements at larger scattering wave vector showed a subsidiary maximum at about $Q = 1.6\ nm^{-1}$.

The results are consistent with a model of the nanoclusters comprising a spherical core of 355±20 $CaHPO_4.2H_2O$ units, density 2.31 g ml^{-1} and radius 2.30±0.05 nm, surrounded by 49±4 peptide chains with a partial specific volume of 0.7 cm^3g^{-1}, forming a tightly packed shell with an outer radius of 4.04±0.15 nm.

Subsequent contrast variation neutron scattering and X-ray scattering measurements on smaller and larger nanoclusters indicate that the shell has a constant thickness, but the core size can be varied. The shell thickness cannot be varied by addition of up to 8M urea and the conformation of the peptide in the shell and in free solution appears to be mostly non-regular, as measured by far UV CD spectroscopy.

The phosphopeptide is able to arrest the process of growth of the precipitating phase of calcium phosphate at its earliest stages. The ability of casein to form nanoclusters in milk suggests a more general mechanism for avoiding pathological calcification and regulating calcium flow in tissues and biological fluids exposed to or containing high concentrations of calcium.

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A Flexible, Integrated Approach to *In Situ* Resolving X-Ray Diffraction Studies of Polymer Systems

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DualAXIS is a fully integrated system developed around a CCD-based area detector, which allows control over sample environment, image processing, and data analysis through a dedicated PC interface. It has been specifically designed for time resolving *in situ* SAXS (Small-Angle X-ray Scattering) and WAXS (Wide-Angle X-ray Scattering) experiments. The most common mode of operation involves subjecting polymer systems to controlled shear deformations, which simulate industrial processing conditions. A small volume multipurpose shear cell has been designed as a sample mount to operate in the temperature range 25-250°C with gas cooling at a rate of 50°C/sec. The software is window based and runs in a Windows NT environment. It contains both experimental control and data analysis. All aspects of the sample environment including the shear rate and temperature can be programmed. The system allows data to be accumulated in a variety of ways with subsequent or in-line analysis. Experiments may be written in the form of a text command file to allow sequences to run without user intervention for prolonged periods. A variety of flexible analysis tools are provided to facilitate measurement of quantitative structural parameters, orientation, and crystallisation in a defined and automated manner.

Collagen Fibril Compaction Accompanies the Acquisition of Transparency in Developing Chick Corneas

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To study some ultrastructural aspects of developing chick corneas we performed a synchrotron X-ray

diffraction analysis of 22 specimens obtained daily from developmental day 10 through day 19. Before day 12 of development in chicks we were unable to detect a meridional X-ray diffraction pattern from cornea. Neither were we able to record a first-order equatorial X-ray reflection at this time. Normally, these reflections are present in corneal X-ray patterns, arising from, respectively, the periodic axial electron density of fibrillar collagen and the lattice-like arrangement of the fibrils. By day 12 of development we could detect the third- and fifth-order meridional reflections (indicating increased amounts of collagen) and a first-order equatorial reflection (implying that more collagen was regularly arranged). The third- and fifth-order meridional reflections became more intense as the tissue matured, suggestive of a continued deposition of fibrillar collagen, and the scattering angle of the interfibrillar maximum increased, suggesting that regularly arranged collagen was becoming more closely packed with maturation. In embryonic chick corneas, the establishment of an orderly, fairly compacted matrix of collagen fibrils may be one of the main events underlying the acquisition of corneal transparency.

Micro-SAXS and Stress / Strain Measurements during the Tensile Deformation of Single Struts of an Elastomeric Polyurethane Foam

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A micro-deformation stage based on a piezoelectric crystal actuator and capable of measuring the force applied to micron-sized polymeric samples is described. Laboratory stress/strain and stress relaxation measurements on a single strut of an elastomeric polyurethane foam have been conducted for the first time. Using this device, micro small-angle X-ray scattering patterns have been collected on the microfocus beamline at the European Synchrotron Radiation Facility, simultaneously with strain and force measurements, during the time-resolved tensile deformation of a single foam strut.

Structural Changes in Tropomyosin and Troponin during Ca^{2+}-Activation of Actin Filaments in Frog Muscle

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Muscular activity is generated when nerve action potentials, propagated into the muscle via the T-tubular system, cause the release within the sarcoplasm of Ca^{2+} ions [1]. These ions in turn attach to the troponin-C component of the troponin complex on the thin, actin-containing, muscle filaments. Each troponin complex interacts via a 40 nm long tropomyosin molecule with seven actin monomers along one strand of the long-pitch actin helix. Studies in the early 1970s [2-4] suggested that the structural effect of calcium binding to troponin is to change the position of the tropomyosin strands within the "groove" of the actin filament helix, thus uncovering or altering the site on actin to which myosin heads bind in order to produce force and movement. This way of controlling the actin-myosin interaction was soon christened the "Steric Blocking Model". Since that time the atomic structure of the actin monomer and filament have been described [5], and further insights into the tropomyosin shift have been obtained both by X-ray fibre diffraction [5] and by electron microscopy and 3D reconstruction [6]. Unfortunately all of these approaches ignored the possible structural implications of the troponin complex on X-ray diffraction or EM data [7]. Troponin has an axial repeat along the actin filaments of about 38.5 nm which is different from the helical repeats (2.75 nm axial translation, about 2 x 37 nm pitch) of the actin filament. We have established a diffraction modelling program that properly includes the contribution from the troponin complex. Unfortunately the full atomic structure of

the troponin complex has not yet been determined. It is possible that this complex may radically change its conformation when Ca^{2+} ions are bound. New results will be presented using a plausible troponin shape, but to date the implication is that even with the troponin taken into account, an azimuthal shift of tropomyosin as in the original "Steric Blocking Model" may be necessary.

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Materials for the Millennium: How Synchrotron Radiation is being used to Research Polymers for the 21st Century

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Polymers have become an essential part of modern-day life. They have found application in a wide range of areas; including packaging materials such as carrier bags, through to more esoteric uses such as artificial heart valves. At Daresbury, the Non Crystalline Diffraction (NCD) facility has been used to examine many aspects of polymer science. Physical measurements have previously been performed in isolation on polymers to try to understand their properties. With the advent of simultaneous multiple techniques developed at Daresbury, it is now possible to combine these physical measurements with data from both Small- and Wide-Angle X-Ray Scattering (SAXS and WAXS) to better understand the physical processes taking place. For example, the rheological behaviour of polymers is better understood when the structural information is clear. This is also true for processing. Study has included *in situ* experiments examining real industrial processes such as extrusion, by which many plastic products are manufactured. The work carried out at Daresbury has led to a better understanding of polymer crystallisation on the academic level and better polymer products on the commercial level.

X-ray and Neutron Reflectivity of Thin Block Copolymer Films

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Diblock copolymers exhibit different microphase-separated morphologies including spheres, hexagonally packed cylinders and lamellar structures. The relative volume fraction of each block along with the Flory-Huggins interaction parameter (χ) and the degree of polymerisation dictates the phase separation and, thus, the lamellar spacing (d-spacing) in the copolymers. Blends of diblock copolymers with homopolymers have been the subject of many studies. Here our study is based upon the work by Winey and co-workers. They investigated the variation of the block copolymer A-B (in the bulk) morphologies upon addition of homopolymer A or B as a blend. In these blends, the free energy is reduced when the homopolymers segregate to the appropriate domains of the ordered structure reducing the number of unfavourable segmental A/B contacts. The microdomains swell in order to accommodate the homopolymers resulting in transitions from one type of microstructure to another in the process. In this study blends of commercially available asymmetric diblock polystyrene-polybutadiene (PS-b-PB) copolymer (MW=83,500 $gmol^{-1}$) with homopolymer (deuterated and hydrogenated PS) and a symmetric diblock polystyrene-poly(dimethylsiloxane) (PS-b-PDMS) (MW=1,000,000 $gmol^{-1}$) have been investigated. Solutions of PS-b-PB blended with 20wt% PS (h-PS or d-PS) and PS-b-PDMS were

prepared in toluene and used to spin-cast thin films at two different spin-speeds (1500, 3000rpm), onto polished silicon wafers, covered with a native oxide layer. Other samples were produced by simply casting the blends into a mould. The morphology of the thin films was studied using X-ray and neutron scattering techniques. Small-Angle X-ray Scattering (SAXS) was used to investigate the morphology of the cast samples and was conducted on station 2.1 of the Daresbury SRS. The deuterated thin films prepared from the blends were examined using the reflectometer CRISP at ISIS, Rutherford and the reflectometer on station 16.2 at Daresbury SRS. From the SAXS studies, a lamellar morphology is detected in the blends of PS-b-PB with h-PS, as does the bulk sample. The intensity of the scattering increases for the blends due to the addition of the homopolymer and therefore the change of the polystyrene volume fraction in the diblock (*i.e.* volume fraction ratio tends to 50/50). The results from neutron and X-ray reflectivity experiments, were combined to determine the number of layers in the film and the effect of the processing techniques on these layers. Unfortunately for the PS-b-PDMS system, the same investigations were made, but due to the high MW and high d-spacing, the instruments did not have the resolution to produce valuable data at the time. However, further investigations will be conducted on the same polymer system having a lower molecular weight.

Phase Separation Behavior in Commercial Al-Li Alloys. A RT-SAXS Characterization

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We present novel results on the phase separation occurring in commercial aluminum-lithium alloys. Quenching the samples from a high temperature state down to a low temperature one, phase separation occurs, leading to the formation of a minority phase, which is finely dispersed in the matrix. The kinetics of the growing of the segregated clusters is followed by means of the real time SAXS technique. Results obtained up to now indicate the occurrence of a bimodal growth behavior in the low temperature regime, while SR-SAXS measurements seem to indicate that in the high temperature regime only a single growth mechanism determines the overall development of morphology. Various analysis approaches are presented in order to extract as much information as possible from experimental results. Simple invariant analysis leads to Avrami plots, which show the above-mentioned behaviour. Moreover, data have also been analyzed by fitting the whole scattering curve by means of a structural model.

α-Tocopherol Induces Two Crystal Phases and One Inverted Hexagonal Phase in Aqueous Dispersions of Dipalmitoylphosphatidylethanolamine

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The effect of α-tocopherol on the structure and thermotropic phase behaviour of aqueous dispersions of dipalmitoylphosphatidylethanolamine in mixtures containing 0, 2.5, 5, 10 and 20mol% α-tocopherol was examined using synchrotron X-ray diffraction methods. Dispersions were equilibrated for 24 hours at a temperature below 30°C before measurement. Pure phospholipids only underwent gel to liquid-crystalline phase transition in the initial heating scan. α-Tocopherol-rich domains, however, were observed in all codispersions of phospholipids with α-tocopherol examined and were assigned on the basis of an increase in scattering intensity of the phase in proportion with the concentration of α-tocopherol in the mixture. In the initial heating scan, α-tocopherol-rich domains, characterised by broad lamellar repeat spacings (5.0 nm at 55°C) in the small-angle region, appear at about 40°C and increase in scattering intensity with increasing temperature. The broad lamellar repeat spacings from the α-tocopherol-rich domains were replaced by an inverted hexagonal structure a few degrees below the gel to liquid-crystalline phase transition temperature of the pure phospholipid at 66°C. The intensity and repeat spacing of the inverted hexagonal phase increased with increasing temperature up to the appearance of

the liquid-crystalline phase. Then the intensity of the inverted hexagonal phase began to decrease with increasing temperature up to the disappearance of the gel phase. The repeat spacing of the inverted hexagonal phase remained relatively constant when coexisting with the liquid-crystalline phase, but, with increasing temperature, the scattering intensity increased at the expense of the lamellar liquid-crystalline phase. Two types of crystal phases Lc1 and Lc2 were observed in all codispersions containing α-tocopherol. The Lc1 is characterised by the first four orders of the sharp diffraction indexing a d-spacing ratio 1:1/2:1/3:1/4 in small-angle region with multiple diffractions in the wide-angle region, and Lc2 is characterised by the broad lamellar diffraction in the small-angle region with multiple diffractions in wide-angle region. Static X-ray diffraction indicates that the stoichiometry of phospholipid:α-tocopherol in Lc1 and Lc2 is about 4:1. In cooling scans performed immediately after heating scans, no crystal phases were observed in all codispersions. The results indicate that during the incubation, α-tocopherol molecules in the gel phase concentrate and form the crystal phase domain Lc1 which has a stoichiometry of 4:1 for phospholipid: α-tocopherol. With increasing temperature the hydrocarbon chains of the domain are tilted at more than 35° to the bilayer normal and transform into the Lc2 domains which transform into the inverted hexagonal phase at higher temperature. When cooling down the inverted hexagonal phase directly transforms into gel phase.

Forthcoming Meetings

9th Annual Fibre Diffraction and Non-Crystalline Diffraction Workshop

June 26-28, 2000, University of Sheffield

(Organised by Mark Shotton, Richard Denny and Trevor Forsyth)

[For further information and registration, see the web pages at http://www.dl.ac.uk/SRS/CCP13 or contact m.shotton@dl.ac.uk]

10th London Muscle Conference

September 15, 2000, Imperial College, London

(Organised by John Squire, Nancy Curtin and Pradeep Luther)

[Details from Prof. John Squire j.squire@ic.ac.uk, 0207 594 3185]

3rd Alpbach Workshop on Fibrous Proteins: "Coiled-Coils, Collagen and Co-Proteins"

September 16-21, 2001, Boglerhof Hotel, Alpbach, Austria

(Organised by David Parry, John Squire and Bob Goldman)

[Details from Prof. John Squire j.squire@ic.ac.uk, 0207 594 3185]

DARTS Bursaries

DARTS at Daresbury Laboratory has funded several bursaries for PhD students to attend the 9th Annual Fibre Diffraction and Non-Crystalline Diffraction Workshop at the University of Sheffield (see above and inside back cover). These bursaries will cover the cost of accommodation and registration and may include a contribution to travelling expenses. An application for a bursary can be made through the web pages at http://www.dl.ac.uk/SRS/CCP13

All bursary applications must be accompanied by the submission of a poster abstract to the Workshop.